PCT/EP 99 / 07405

Europäisches Patentamt | European Patent Office | Office européen des brevets

4

Bescheinigung | Certificate | Attestation

EP99/7405

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

98203280.7

09/806178

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts:
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN
THE HAGUE, 08/11/99
LA HAYE, LE

PCT/EP 99 / 07405

Europäisches Patentamt
European Patent Office
Office européen des brevets

5

E

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.
Application no.
Demande n°: 98203280.7

Anmeldetag
Date of filing
Date de dépôt: 29/09/98

Anmelder
Applicant(s)
Demandeur(s)
Leuven Research & Development V.Z.W.
3000 Leuven
BELGIUM

Bezeichnung der Erfindung
Title of the invention
Titre de l'invention
New method for the treatment of ischemic stroke

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat State Pays	Tag Date Date	Aktenzeichen File no. Numéro de dépôt

Internationale Patentklassifikation
International Patent classification
Classification internationale des brevets
/

Am Anmeldetag benannte Vertragstaaten
Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du dépôt

Bemerkungen
Remarks
Remarques

PATENT APPLICATION

NEW METHOD FOR THE TREATMENT OF ISCHEMIC STROKE

The present invention relates to a new method for the treatment of focal ischemic cerebral infarction (ischemic stroke), based on reduction of the activity of α_2-antiplasmin, the physiological plasmin inhibitor of the fibrinolytic system. Focal ischemic cerebral infarction occurs when the arterial blood flow to a specific region of the brain is reduced below a critical level resulting in neuronal cell death. We have, surprisingly found, contrary to earlier believe, that the cerebral infarct size caused by occlusion of the middle cerebral artery (MCA) is proportional to the concentration of circulating α_2-antiplasmin and that administration of a compound that reduces the activity of α_2-antiplasmin, such as the Fab fragments of antibodies directed against α_2-antiplasmin, significantly reduces the infarct size associated with MCA occlusion.

The fibrinolytic system includes a proenzyme, plasminogen (Plg), which is converted by plasminogen activators (PA) to the active enzyme plasmin, which in turn digests fibrin to soluble degradation products. Inhibition of the fibrinolytic system takes place at the level of the plasminogen activators (mainly by plasminogen activator inhibitor-1, PAI-1) and of plasmin (mainly by α_2-antiplasmin, α_2-AP) (1).

Neuronal degeneration in central nervous system (CNS) diseases such as stroke, epilepsy and Alzheimer's disease is thought to be stimulated by an excess of the excitatory amino acid glutamate (2). Injection of glutamate agonists in the CNS indeed induces hippocampal neuronal cell death similar to that observed in neurodegenerative diseases (3). Excitotoxin-induced neuronal degeneration is mediated by tissue-type plasminogen activator (t-PA) (4). Consistent with this observation, mice deficient in t-PA are resistant to, and infusion of PAI-1 protects against excitotoxin-mediated hippocampal neuronal degeneration (4-6). Furthermore, deficiency of Plg, the zymogen substrate of t-PA, and infusion of α_2-AP, protect mice against excitotoxin-induced hippocampal neuronal death (5). It has been proposed that plasmin-mediated degradation of laminin sensitizes hippocampal neurons to cell death by disrupting neuron-extracellular matrix interaction (7).

Wang et al (8) recently demonstrated that neuronal damage after focal cerebral ischemia induced by transient occlusion of the middle cerebral artery was also reduced in mice with t-PA deficiency and exacerbated by t-PA infusion. Thus the plasminogen system may be involved both in establishing a

cerebral ischemic infarct and in its extension during thrombolytic therapy. We recently demonstrated that the neurotoxic effect of t-PA on persistent focal cerebral ischemia also occurred with other thrombolytic agents, including streptokinase and staphylokinase (9). Thus, in those patients with persistent cerebral arterial occlusion, thrombolytic therapy for ischemic stroke may cause infarct extension, which would not only partially offset the established overall beneficial effect of arterial recanalization (10, 11), but indeed be harmful to a subgroup of patients. Because it is not possible to distinguish between patients who will and those who will not achieve cerebral arterial recanalization with thrombolytic therapy, the development of specific conjunctive strategies to counteract the neurotoxic effects of thrombolytic agents on persisting focal cerebral ischemia appear to be warranted.

Although it is assumed that neuronal injury during focal ischemia in the brain occurs primarily as a result of accumulation of excitotoxins such as glutamates, the role of plasmin-mediated laminin degradation or alternative mechanisms in the pathogenesis of cortical neuronal cell death has not been demonstrated. In order to delineate the contribution of individual components of the plasminogen (fibrinolytic) system on focal cerebral ischemic infarction, we have quantitated infarct size produced by ligation of the left middle cerebral artery (MCA) in mice with targeted inactivation of the genes encoding Plg, its activators t-PA or u-PA, or the fibrinolytic inhibitors PAI-1 or α_2-AP. In addition, the effects of adenoviral transfer of the *t-PA* and *PAI-1* genes and of infusion of human α_2-AP on cerebral infarction were studied.

Whereas the findings of Strickland et al, that t-PA deficiency protects against focal cerebral ischemic infarction were fully confirmed, and extended by the observation that PAI-1 deficiency resulted in significantly larger infarct sizes, the observation that Plg deficiency protects against excitotoxin induced neuronal cell death could not be confirmed. Instead we found that focal cerebral infarct size was significantly larger in mice with Plg deficiency and conversely, significantly smaller in mice with α_2-AP deficiency.

In aggregate, our findings indicate that plasminogen system components affect focal cerebral ischemic infarct size at two different levels: 1) reduction of t-PA activity (*t-PA* gene inactivation or

PAI-1 gene transfer) reduces, while its augmentation (*t-PA* gene transfer or *PAI-1* gene inactivation) increases infarct size, and 2) reduction of Plg activity (*Plg* gene inactivation or α_2-AP injection) increases, while its augmentation (*α_2-AP* gene inactivation or α_2-AP neutralization) reduces infarct size. The findings are incompatible with a unique linked pathway in which t-PA-mediated plasmin generation would lead to neuronal cell death, but suggests two independent (t-PA mediated and Plg-mediated, respectively) mechanisms operating in opposite direction.

The internally consistent observations with α_2-AP were unexpected but are most relevant for the treatment of ischemic stroke. Firstly a correlation was found between infarct size and genotype with heterozygotes displaying infarct sizes between those of the wild type and homozygous phenotypes. Secondly, bolus injection of human α_2-AP (hα_2-AP) in α_2-AP$^{-/-}$ mice caused a dose-related infarct expansion. Finally, and importantly, Fab fragments from affino-specific polyclonal rabbit anti-hα_2-AP antibodies, given intravenously 40 min after occlusion of the MCA, significantly reduced the cerebral ischemic infarct size. This observation suggests a potential avenue to counteract focal ischemic infarction with the use of α_2-AP inhibitors (e.g. neutralizing monoclonal antibodies or compounds neutralizing α_2-AP activity). The concentration of α_2-AP in human plasma is 1 μM (12), corresponding to a total body pool of approximately 500 mg. An equivalent dose of a monoclonal Fab fragment would be approximately 400 mg, which is high but not excessive for single therapeutic administration. Furthermore, the observation that infarct size is proportional to the α_2-AP level (derived from the gene dose effect and the dose-response) suggests that a partial reduction of the plasma level might have a significant beneficial effect.

The present invention will be demonstrated in more detail in the following examples, which are however not intended to be limiting to the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 to 3 are histograms comparing the volume (in mm^3) of focal cerebral ischemic infarcts after ligation of the middle cerebral artery (MCA) in mice. The data represent mean values and the vertical bars SEM, with the number of experiments given in the columns.

Figure 1: Effect of deficiency of plasminogen system components (genotype in abscissa) on focal ischemic cerebral infarct size (in mm^3).

WT: wild type (pooled values of 50% C57BL6/50% S129, 100% C57BL6 and 100% S129 genetic background).

Figure 2: Effect of adenoviral transfer of the *t-PA* or *PAI-1* genes on focal ischemic cerebral infarct size in t-PA or PAI-1 deficient mice, respectively.

Figure 3: Effect of α_2-AP on focal ischemic cerebral infarct size.

A. Effect of α_2-AP genotype on cerebral infarct size.

B. Effect of injection of hα_2-AP or of hα_2-AP followed by anti-hα_2-AP Fab fragments on cerebral infarct size.

EXAMPLE I: MURINE CEREBRAL ISCHEMIC INFARCTION MODEL

All mice included in the present study were generated and bred at the Specific Pathogen Free Facility of the Center for Transgene Technology and Gene Therapy, Campus Gasthuisberg, K.U.Leuven. Gene inactivation was obtained by homologous recombination in embryonic stem cells targeting the genes encoding tissue-type plasminogen activator (t-PA) (13), urokinase-type plasminogen activator (u-PA) (13), plasminogen activator inhibitor-1 (PAI-1) (14,15), plasminogen (Plg) (16), or α_2-antiplasmin (α_2-AP) (17), as previously described. Mice with inactivated genes encoding u-PA receptor (u-PAR) (18) were not included because of the normal results obtained with u-PA deficient mice.

Animal experiments were conducted according to the guiding principles of the American Physiological Society and the International Committee on Thrombosis and Haemostasis (19).

Focal cerebral ischemia was produced by persistent occlusion of the MCA according to Welsh et al. (20). Briefly, mice of either sex, weighing 20 to 30 g, were anesthetized by intraperitoneal injection of ketamine (75 mg/ml, Apharmo, Arnhem, The Netherlands) and xylazine (5 mg/ml, Bayer, Leverkusen, Germany). Atropine (1 mg/kg; Federa, Brussels, Belgium) was administered

intramuscularly, and body temperature was maintained by keeping the animals on a heating pad. A "U" shape incision was made between the left ear and left eye. The top and backside segments of the temporal muscle were transsected and the skull was exposed by retraction of the temporal muscle. A small opening (1 to 2 mm diameter) was made in the region over the MCA with a hand-held drill, with saline superfusion to prevent heat injury. The meningae were removed with a forceps and the MCA was occluded by ligation with 10-0 nylon thread (Ethylon, Neuilly, France) and transsected distally to the ligation point. Finally, the temporal muscle and skin were sutured back in place.

AdCMVt-PA, AdCMVPAI-1 or AdRR5 were given as an intravenous bolus of 1.3 x 10^9 plaque forming units (p.f.u.) 4 days before ligation of the MCA. Human α_2-AP (hα_2-AP) was given intravenously divided in 2 injections, given 1 min before and 30 min after ligation of the MCA respectively. Fab fragments were injected intravenously as a bolus, 10 min after the second hα_2-AP injection.

The animals were allowed to recover and were then returned to their cages. After 24 hours, the animals were sacrified with an overdose of Nembutal (500 mg/kg, Abbott Laboratories, North Chicago, IL) and decapitated. The brain was removed and placed in a matrix for sectioning in 1 mm segments. The sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride (TTC) in saline (21), incubated for 30 min at 37°C, and placed in 4 % formalin in phosphate buffered saline. With this procedure, the necrotic infarct area remains unstained (white) and is clearly distinguishable from stained (brick red) viable tissue. The sections were photographed and subjected to planimetry. The infarct volume was defined as the sum of the unstained areas of the sections multiplied with their thickness.

The recombinant adenoviruses AdCMVt-PA and AdCMVPAI-1 were generated by homologous recombination in 293 cells essentially as previously described (22). For AdCMVt-PA, an *Xba*I-fragment of the plasmid pSTEt-PA encoding wild type human t-PA was ligated into XbaI-digested pACCMVpLpA (23) to produce pACCMVt-PA. The adenovirus precursor pACCMVPAI-1 was generated by ligating the 1.4-kb *Eco*RI/*Bgl*II fragment of pPAI-1RBR containing the entire coding

sequence of human PAI-1 in EcoRI/BamHI-digested pACCMVpLpA. In these plasmids, the t-PA and PAI-1 cDNA are positioned between the human cytomegalovirus immediate-early enhancer/promoter and the SV40 t-antigen intron/polyadenylation signal to form a complete transcriptional unit.

Monolayer cultures of 293 cells (24) were cotransfected with 10 µg of pACCMVt-PA or pACCMVPAI-1 and 5 µg of pJM17 (22), a plasmid containing a full-length adenovirus 5 *dl*309 genome. Homologous recombination between these plasmids results in the formation of recombinant viral genomes in which the adenovirus E1 region is replaced by the respective *t-PA* or *PAI-1* transgenes. Replication of the recombinant viruses in cultured 293 cells is supported by *E1A* gene products supplied *in trans* from a copy of *E1* integrated into the 293 cell genome.

After transfection, recombinant viral plaques were harvested and amplified as described (25). The identity of recombinant viruses was determined by restriction analysis and Southern blotting of viral DNA prepared from productively infected 293 cells. The recombinant adenovirus AdRR5, which lacks an inserted gene in the E1 position, was generated from pACRR5 and pJM17 in the same manner and was used as a control adenovirus (26,27). Recombinant viruses were replaqued to ensure clonal identity before further use. Large scale production of recombinant adenovirus was performed as described (25). Purified virus was supplemented with 0.1 mg/ml sterile bovine serum albumin (BSA), snap frozen in liquid nitrogen and stored at –80°C until use. The titer of infectious viral particles in purified stocks was determined by plaque assay on monolayers of 293 cells with 1 hour of adsorption at 37°C. Purified viral stocks of $>10^{10}$ plaque forming units (pfu) per ml were routinely obtained. The kinetics and organ distribution of t-PA and PAI-1 expression following adenoviral transfer by intravenous bolus injection have been described elsewhere (28,29).

Human α_2-AP was prepared from fresh frozen plasma as previously described (30).

Polyclonal antisera were raised in rabbits by subcutaneous injection of 200 µg purified human α_2-AP suspended in complete Freund's adjuvant, followed at two biweekly intervals by injection of the antigen suspended in incomplete Freund's adjuvant. Serum was obtained by repeated ear vein

puncture. Pooled sera were chromatographed on Protein-A Sepharose (0.5 ml serum per ml wet gel), equilibrated with 0.1 M Tris.HCl, pH 8.1 and IgG eluted with 0.1 M glycine.HCl, pH 2.8, yielding approximately 10 mg protein per ml serum. Affino-specific antibodies were obtained from the dialyzed IgG pool by chromatography on a CNBr-activated Sepharose column substituted with human α_2-AP (2.5 mg/ml wet gel) and eluted with 0.1 M glycine.HCl, pH 2.8, yielding approximately 0.1 mg specific IgG per mg applied. Fab fragments were obtained from the affino-specific IgG by digestion with papain. Therefore IgG was dissolved to a concentration of 5 mg/ml and digested with 1 percent (w/w) papain in the presence of 50 mM cysteine, 1 mM EDTA, 0.1 M phosphate buffer, pH 7.0 for 5 hours. The reaction was arrested by addition of iodoacetamide to a final concentration of 75 mM. After dialysis the mixture was purified on a protein A.Sepharose column equilibrated with PBS. Fab concentration was determined by ELISA calibrated against an IgG standard. SDS gel electrophoresis essentially revealed homogeneous Fab fragments (not shown).

The data are represented as mean ± SEM of n determinations. The significance of differences was determined using analysis of variance followed by Fisher's PLSD test, using the Statview software package.

EXAMPLE II: CEREBRAL ISCHEMIC INFARCT SIZE IN MICE WITH TARGETED INACTIVATION OF GENES ENCODING PLASMINOGEN SYSTEM COMPONENTS

Ligation of the left MCA induced a cerebral infarct with a volume of 7.6 ± 1.1 mm^3 (n= 11) in wild type mice with a mixed (50%) S129 and (50%) C57BL/6 genetic background, of 9.3 ± 2.7 mm^3 (n= 6) in inbred C57BL/6 mice and of 6.4 ± 1.3 mm^3 (n= 6) in inbred S129 mice (p= NS versus mixed background, results not shown).

Inactivation of the *t-PA* gene was associated with a significant reduction of infarct size to 2.6 ± 0.80 mm^3 (n= 11), (p< 0.0001 vs wild type mice), whereas inactivation of the *u-PA* gene had no effect on infarct size (7.8 ± 1.0 mm^3, n= 8, p= NS vs wild type). Inactivation of the *PAI-1* gene

was associated with a significant increase in infarct size (16 ± 0.52 mm^3, n= 6, p< 0.0001 vs wild type) (Figure 1). In mice with inactivated *Plg* genes, cerebral infact size was significantly larger than in wild type mice (12 ± 1.2 mm^3, n=9, p=0.037 vs wild type), whereas, conversely, in α_2-*AP* gene deficient mice, infarct size was markedly reduced (2.2 ± 1.1 mm^3, n= 7, p= 0.0001 vs wild type) (Figure 1).

EXAMPLE III: EFFECT OF *t-PA* AND *PAI-1* GENE TRANSFER ON CEREBRAL INFARCT SIZE

Injection of 1.3×10^9 p.f.u. of AdCMVt-PA in 6 t-PA$^{-/-}$ mice 4 days before MCA ligation was associated with a cerebral infarct size of 6.0 ± 1.3 mm^3, significantly larger than the infarcts in 5 t-PA$^{-/-}$ mice injected with the control virus AdRR5 (1.8 ± 0.63, p= 0.028) (Figure 2A). Conversely, injection of 1.3×10^9 p.f.u. of AdCMVPAI-1 in 5 PAI-1$^{-/-}$ mice was associated with a cerebral infarct size of 10 ± 1.4 mm^3, significantly smaller than the infarcts in 5 PAI-1$^{-/-}$ mice injected with the control virus AdRR5 (13 ± 1.0 mm^3, p= 0.019) (Figure 2B).

EXAMPLE IV: EFFECT OF α_2-ANTIPLASMIN ON CEREBRAL INFARCT SIZE

Cerebral infarct size correlated with α_2-*AP* gene dose, corresponding to 11 ± 2.0, 4.9 ± 2.0 and 2.2 ± 1.1 mm^3 in wild type, heterozygous and homozygous deficient mice, respectively (Figure 3A). Injection of human α_2-AP in groups of 4 α_2-AP$^{-/-}$ mice increased the infarct size to 13 ± 2.5 mm^3 (n= 4) with a 1 mg total dose and to 11 ± 1.5 mm^3 (n= 6) with a 0.2 mg total dose. Injection of 1.7 mg affino-specific Fab against human α_2-AP in mice given 0.2 mg human α_2-AP reduced the cerebral infarct size to 5.1 ± 1.1 mm^3 (n= 7, p= 0.0040 vs 0.2 mg human α_2-AP) (Figure 3B).

The above examples show that reduction of α_2-AP activity (reduced α_2-*AP* gene expression or reduction of circulating α_2-AP with inhibitors) reduces focal cerebral ischemic infarct size, such as encountered during ischemic stroke.

REFERENCES

1. Collen D. Staphylokinase: a potent, uniquely fibrin-selective thrombolytic agent. Nature Medicine 1998; 4: 279-284.

2. Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative disorders. Science 1993; 262: 689-695.

3. Coyle JT, Molliver ME, Kuhar MJ. In situ injection of kainic acid: a new method for selectively lesioning neuronal cell bodies while sparing axons of passage. J Comp Neurol 1978; 180: 301-323.

4. Tsirka S, Gualandris A, Amaral DG, Strickland S. Excitotoxin induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. Nature 1995; 377: 340-344.

5. Tsirka S, Rogove AD, Bugge TH, Degen JL, Strickland S. An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. J Neurosci 1997; 17: 543-552.

6. Tsirka S, Rogove AD, Strickland S. Neuronal cell death and t-PA. Nature 1996; 384: 123-124.

7. Chen ZL, Strickland S. Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. Cell 1997; 91: 917-925.

8. Wang YF, Tsirka SE, Strickland S, Stieg PE, Soriano SG, Lipton SA. Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. Nature Medicine 1998; 4: 228-231.

9. Nagai N, Vanlinthout I, Collen D. Comparative effects of tissue-type plasminogen activator, streptokinase and staphylokinase on cerebral ischemic infarction and pulmonary clot lysis in hamster models. Submitted.

10. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995; 333: 1581-1587.

11. Hacke W, Kaste M, Fie[illegible]n C, Toni D, Lesaffre E, von Kummer R, Boysen G, Bluhmki E, Haxter G, Mahagne MH, Hennerici M, for the ECASS Study Group. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke: the European Cooperative Actue Stroke Study (ECASS). J Am Med Ass 1995; 274: 1017-1025.

12. Collen D, Wiman B. Turnover of antiplasmin, the fast-acting plasmin inhibitor of plasma. Blood 1979; 53: 313-324.

13. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degen J, Bronson R, De Vos R, van den Oord J, Collen D, Mulligan R. Physiological consequences of loss of plasminogen activator gene function in mice. Nature 1994; 368: 419-424.

14. Carmeliet P, Kieckens L, Schoonjans L, Ream B, Van Nuffelen A, Prendergast G, Cole M, Bronson R, Collen D, Mulligan RC. Plasminogen activator inhibitor-1 gene-deficient mice. I. Generation by homologous recombination and characterization. J Clin Invest 1993; 92: 2746-2755.

15. Carmeliet P, Stassen JM, Schoonjans L, Ream B, Van den Oord JJ, De Mol M, Mulligan RC, Collen D. Plasminogen activator inhibitor-1 deficient mice. II. Effects on hemostasis, thrombosis and thrombolysis. J Clin Invest 1993; 92: 2756-2760.

16. Poplis VA, Carmeliet P, Vazirzadeh S, Van Vlaenderen I, Moons L, Plow EF, Collen D. Effects of disruption of the plasminogen gene on thrombosis, growth and health in mice. Circulation 1995; 92:2585-2593.

17. Lijnen HR, Okada K, Matsuo O, Collen D, Dewerchin M. α_2-antiplasmin gene-deficiency in mice is associated with enhanced fibrinolytic potential without overt bleeding. Blood (submitted).

18. Dewerchin M, Van Nuffelen A, Wallays G, Bouché A, Moons L, Carmeliet P, Mulligan RC, Collen D. Generation and characterization of urokinase receptor-deficient mice. J Clin Invest 1996; 97: 870-878.

19. Giles AR. Guidelines for the use of animals in biomedical research. Thromb Haemost 1987; 58: 1078-1084.

20. Welsh FA, Sakamoto T, McKee AE, Sims RE. Effect of lactacidosis on pyridine nucleotide stability during ischemia in mouse brain. J Neurochem 1987; 49: 846-851.

21. Bederson JB et al. Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke 1986; 17: 472-476.

22. McGrory WJ, Bautista DS, Graham FL. A simple technique for the rescue of early region 1 mutations into infectious human adenovirus type 5. Virology 1988; 163: 614-617.

23. Gomez-Foix AM, Coats WS, Baque S, Alam T, Gerard RD, Newgard CB. Adenovirus-mediated transfer of the muscle glycogen phosphorylase gene into hepatocytes confers altered regulation of glycogen metabolism. J Biol Chem 1992; 267: 25129-25134.

24. Graham FL, Smiley J, Russel WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J Gen Virol 1977; 36: 59-74.

25. Gerard RD, Meidell RS. Adenovirus vectors. In: Hames BD, Glover D (eds): DNA Cloning – A practical approach: mammalian systems. Oxford, UK, 1995, p 285-307.

26. Alcorn JL, Gao E, Chen Q, Smith ME, Gerard RD, Mendelson CR. Genomic elements involved in transcriptional regulation of the rabbit surfactant protein-A gene. Mol Endocrinol 1993; 7: 1072-1085.

27. Kopfler WP, Willard M, Betz T, Willard JE, Gerard RD, Meidell RS. Adenovirus-mediated transfer of a gene encoding human apolipoprotein A-Iinto normal mice increases circulating high-density lipoprotein cholesterol. Circulation 1994; 90: 1319-1327.

28. Carmeliet P, Stassen JM, Van Vlaenderen I, Meidell RS, Collen D, Gerard RD. Adenovirus-mediated transfer of tissue-type plasminogen activator augments thrombolysis in tissue-type plasminogen activator-deficient and plasminogen activator inhibitor-1-overexpressing mice. Blood 1997; 90: 1527-1534.

29. Carmeliet P, Moons L, [redacted]n R, Janssens S, Lupu F, Collen D, [redacted]rard RD. Inhibitory role of plasminogen activator inhibitor-1 in arterial wound healing and neointima formation. Circulation 1997; 96: 3180-3191.

30. Lijnen HR, Holmes WE, Van Hoef B, Wiman B, Rodriguez H, Collen D. Amino-acid sequence of human α_2-antiplasmin. Eur J Biochem 1987; 166: 565-574.

CLAIMS

What we claim is:

1. The use of compounds that reduce α_2-antiplasmin activity in vivo, for the treatment of focal cerebral ischemic infarction (ischemic stroke).

2. The use of compounds according to claim 1, characterized by the fact that the circulating α_2-antiplasmin concentration is reduced.

3. The use of compounds according to claim 1, characterized by the fact that the circulating α_2-antiplasmin activity is reduced.

4. The use of compounds according to claim 1, in which compounds are α_2-antiplasmin neutralizing antibodies or derivatives thereof.

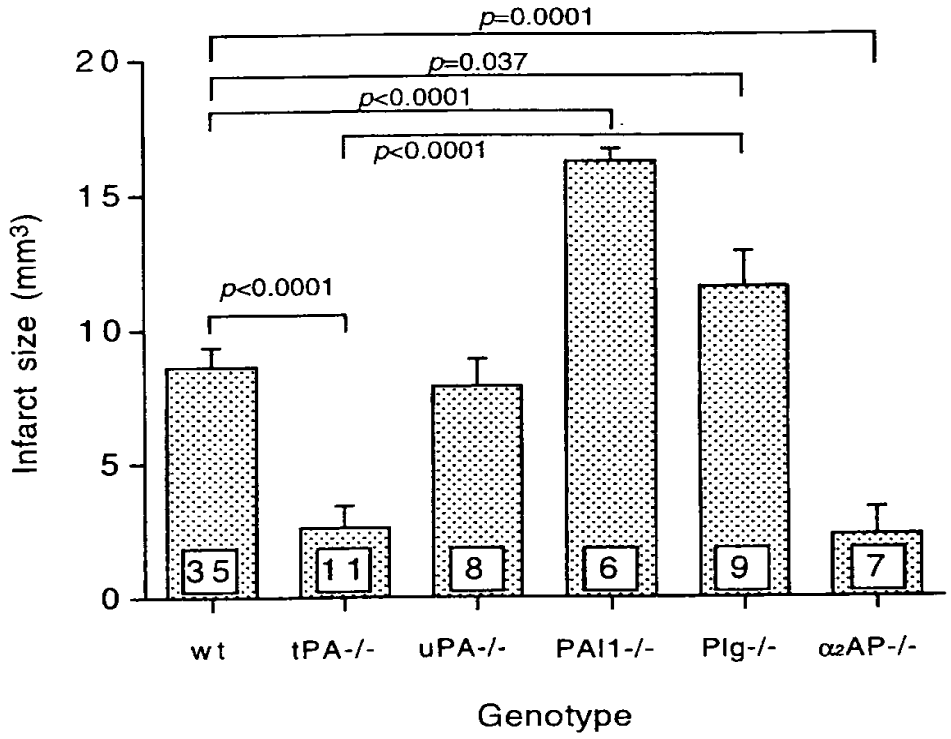
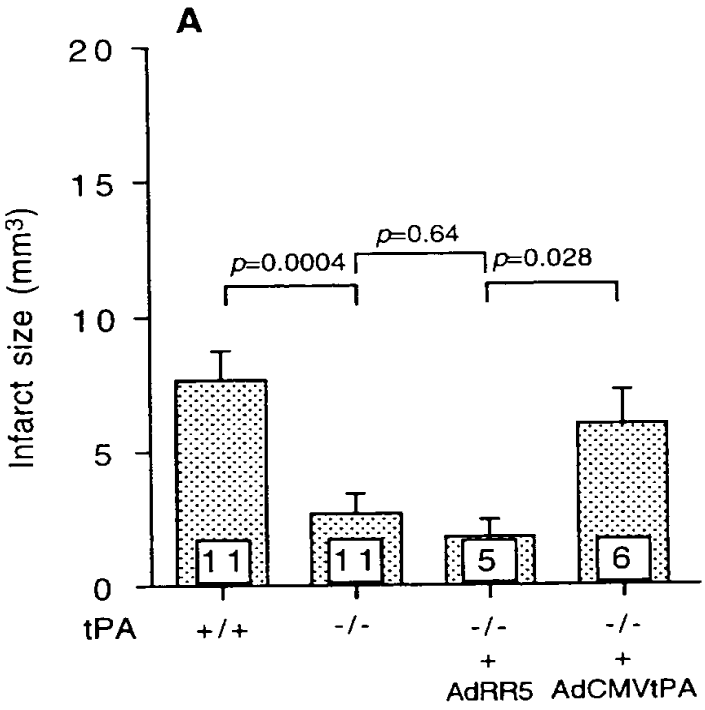


Fig 1


A
20
15
10
5
0
Infarct size (mm3)
p=0.0004
p=0.64
p=0.028
11
11
5
6
tPA
+/+
-/-
-/-
+
AdRR5
-/-
+
AdCMVtPA

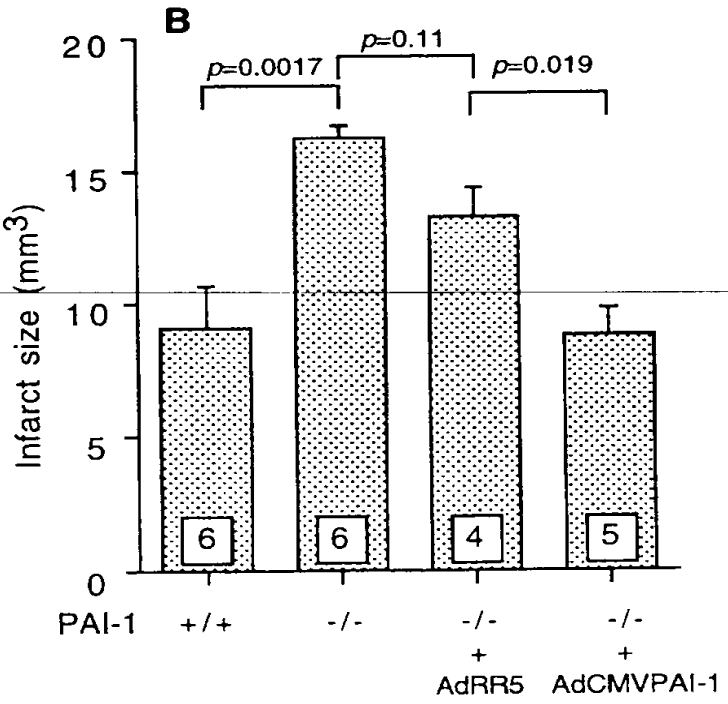
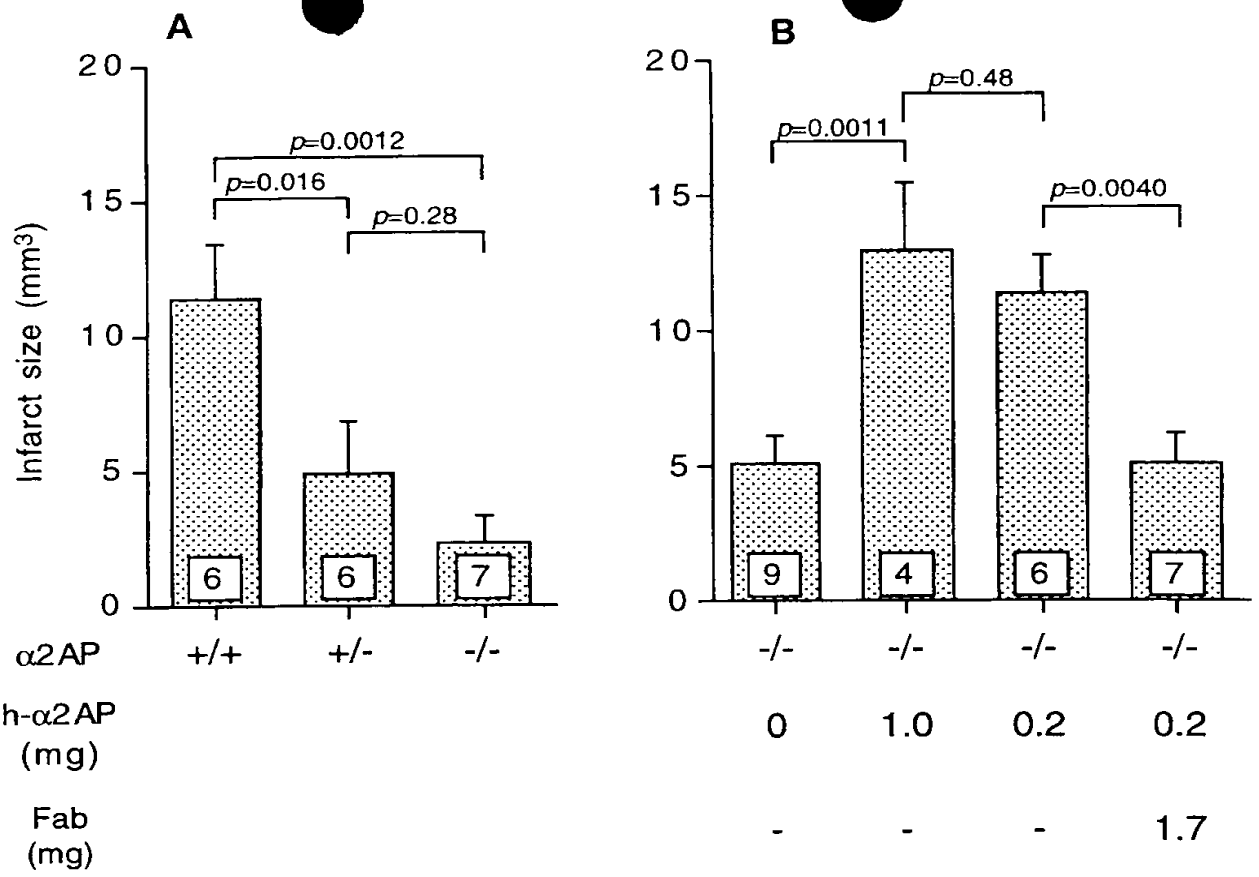

B
20
15
10
5
0
Infarct size (mm3)
p=0.0017
p=0.11
p=0.019
6
6
4
5
PAI-1
+/+
-/-
-/-
+
AdRR5
-/-
+
AdCMVPAI-1

Fig 22


A
B
Infarct size (mm3)
20
15
10
5
0
p=0.0012
p=0.016
p=0.28
6
6
7
p=0.48
p=0.0011
p=0.0040
9
4
6
7
α2AP
+/+
+/-
-/-
-/-
-/-
-/-
-/-
h-α2AP (mg)
0
1.0
0.2
0.2
Fab (mg)
-
-
-
1.7